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ANTIFUNGAL EFFICACY OF GARLIC AND GINGER AGAINST SCLEROTIUM ROLFSII

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ABSTRACT

Uses of synthetic fungicides are cost effective, non-economical, had detrimental effect on health and led to increased resistance and soil pollution. However use of Botanicals and their active compounds were commercialized recently to combat several diseases. Based on this overview, in the present study two botanicals Garlic (Liliaceae) and Ginger (Zingiberaceae) were selected at 1, 2, 3, 4 & 5% and their combination in ratio 1:1, 2:1, 3:1 & viceversa were tested alongwith two well known synthetic fungicides, viz., Bavistin (500, 750 & 1000 ppm) and Dithane M-45(1500, 2000 & 2500 ppm), to check their antifungal efficacy against Sclerotium rolfsii. Garlic at 4 - 5% was 100% effective against S.rolfsii as compared to control which showed 90mm mycelial growth of S. rolfsii with 100% sclerotial formation, but at 3% mycelial growth was inhibited by 43.7 % as compared to 5% ginger (51.5%) while ginger at lower concentration viz., 1- 4% were less effective. But it showed inhibitory effect when applied in combination of garlic; the mixture of garlic: ginger (2:1) reduced radial growth upto 23% and garlic: ginger (3:1) showed strong efficacy against S. rolfsii , while the mixture of garlic: ginger (1:1), ginger: garlic (2:1) was ineffective against S. rolfsii. However Ginger: Garlic (3:1) showed enhanced efficacy inhibiting @ 22.6%. Comparatively synthetic fungicide Dithane - M45 inhibited 100% growth of S. rolfsii while Bavistin failed to inhibit growth of S. rolfsii at all test concentration. Garlic and ginger could be potent alternatives to synthetic fungicides to combat severity of disease caused by S.rolfsii.

KEYWORDS: Synthetic Fungicides, Botanicals, Sclerotium Rolfsii & Inhibition

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INTRODUCTION

Sclerotium rolfsii (Teleomorph: *Athelia rolfsii*) popularly known as white mold is a dreaded soilborne fungi which are responsible for causing numerous crop diseases and can infect more than 500 plant species. The pathogen producing characteristic brown mustard seed like sclerotia (0.5- 2mm) and white mycelial mats infects crown region of stem resulting to wilting and dieback disease. Soybean (*Glycine max*) southern blight disease (*Sclerotium rolfsii*) is highly devastative affecting crop yield varied from 5-20% (Mullen, 2001).

Southern blight is an important disease of soybean in Jharkhand. The disease commonly prevails during reproductive and pod filling stages of the crop plants; during the 2nd fortnight of August- 1st fortnight of September. The affected plants leaves turn brown, dry-up and finally gets wilted and die. The closed observation showed a light brown girdling lesion just above the soil line. The most characteristic sign of the disease was the white fungal mat of mycelia which fans out over and about lesion area of the stem. Fungal mat may also be present on plant debris and on soil surface in the vicinity of an infected plant. The major predisposing factor of this disease is high moisture levels in both soil and dense canopy of the crop plants (ICAR-RCER, Patna news 2012).

Undoubtedly, for management of this disease; use of synthetic fungicides remains an effective measure in this region. But enormous use of chemical pesticides had paved the way towards environmental pollution, proved carcinogenic and developed resistance in microbes. However, several botanicals including garlic and ginger were found to be effectively inhibiting the growth of soilborne and sporulating fungi both *in vivo* and *in vitro* (Suleiman and Abdallah, 2014, Tohamy *et al*., 2002). Moreover several researchers elucidated that combined effect of botanicals or biocides showed strong synergistic efficacy in inhibition of plant pathogenic microbes as when applied in alone (Singh *et al*., 2003, Hanan *et al.,* 2012, Chusri *et al*., 2014, Bhuva and Dixit, 2015). As per disastrous post effect of synthetic fungicides, searching out a potent botanical plant product having ability to inhibit individually and in form of mixture becomes a dire need. Keeping these in view, garlic and ginger extracts at different concentrations; in individual and combinations were selected to see their antifungal efficacy. Synergistic effect of both plants will be evaluated against *S. rolfsii* and compared with the magnitude of effect of synthetic fungicides viz. Bavistin (carbendazim 50 w/p) and Dithane-M 45(Mancozeb 75 w/p) against the test fungus.

MATERIALS AND METHODS

Isolation and Purification of *S. rolfsii*

Southern blight infected Soyabean (Fabaceae) plant was collected from experimental research farm ICAR- RCER- RC, Plandu, Ranchi. Collar regions of the stem showing fungal infestation were cut into small pieces, washed thoroughly with sterilized distilled water and kept in 2% sodium hypochlorite solution for 2 min. After washing with sterilized distilled water, the infected pieces were cut into 3-5 mm in length and kept in moist chamber for induced mycelial growth. Appearance of white mycelial thread were observed after 2 days. Soon after the identification; the purification of the test fungus was done through hyphal tip isolation technique. After transferring in PDA slants the culture was maintained at 25 ± 2^0C.

Plant Materials

Healthy and non-infected bulb of garlic and rhizome of ginger were purchased from local market of Jharkhand. The collected materials were peeled and washed subsequently under tap water and with sterilized distilled water. The rinsed plant parts after shade drying for 24 hrs at room temperature (24 ± 2^0C) were chopped in sterilized mortar and pestle.

Antimycotic Assay by Food Poisoned Technique

Different concentration of freshly macerated plant parts were used viz. 1, 2, 3, 4 and 5% to see their antifungal efficacy against *S. rolfsii* by food poisoned technique. In 100 ml of potato -dextrose -agar media; 1, 2, 3, 4 and 5g of macerated plant material were mixed in 1:1 ratio and steam autoclaved at 121 °C under 15 psi for 15 minutes. The combinations of both plants were also prepared in ratio 1:1(0.5g garlic + 0.5g ginger), 2:1(1.3g garlic+ 0.7g ginger), and 3:1(2.25g garlic+ 0.75g ginger) and vice-versa. Similarly based on recommended doses of Synthetic fungicides such as Carbendazim(1-1.5g/l) and Mancozeb(2g/l), three concentrations of both fungicides were prepared i.e., 500, 750 and 1000 ppm of Carbendazim, and 1500, 2000 and 2500 ppm of Mancozeb were mixed with PDA to check their antifungal property against the test pathogen. 5mm bead were cut from fully grown culture of *S. rolfsii* and inoculated in various treatments as well as in control. The periodical growth of the fungus was observed after every 24 hrs.

Effect of Extracts on Fungal Growth

The radial growth of the test fungus were recorded in terms of percentage inhibition in colony diameter and calculated by formula used by (Pandey *et al*., 1982, Okibo and Nmeka, 2005):

Growth inhibition (I)% = [(DC – DT) / DC] × 100

Where,

DC= average diameter of fungal colony in control, and DT= average diameter of fungal colony in treatment.

RESULTS AND DISCUSSIONS

In the present study two plants extracts *A. sativum* (Garlic) and *Z. officinale* (Ginger) at 1, 2, 3, 4 and 5% concentration, their combination in ratio 1:1, 2:1 and 3:1 and viceversa alongwith comparative effect of synthetic fungicides such as carbendazim and mancozeb were selected to check their antifungal efficacy against *S. rolfsii*; the causal organism of southern blight disease in *Glycine max* (soyabean). Results showed that the aqueous extract of garlic at 4% and 5% was 100% effective against *S.rolfsii* as compared to control which showed 90 mm mycelial growth of *S. rolfsii* with 100% sclerotial formation (shown in Table 1, 2 & 3 and Fig.1 and 2). Although garlic was found ineffective at lower concentration 1-2% but at 3% mycelial growth was inhibited by 43.7 % as compared to 5% ginger (51.5%) while ginger at lower concentration viz., 1- 4% were ineffective against the test pathogen. But it showed strong antifungal efficacy when applied in combination of garlic and ginger; the mixture of garlic(2): ginger(1) reduced radial growth upto 34.1% and garlic(3): ginger(1) inhibited growth of *S. rolfsii* upto 56.6 %, while the mixture of Garlic(1): Ginger(1), Ginger(2): Garlic(1) was not effective against *S.rolfsii*. However Ginger (3): Garlic (1) enhanced efficacy inhibiting the pathogen @ 22.6%. Comparatively synthetic fungicide mancozeb inhibited 100% growth of *S. rolfsii* at all test concentration i.e., 1500, 2000 and 2500 ppm. While carbendazim failed to inhibit growth of *S.rolfsii* at all test concentration viz. 500, 750 and 1000 ppm.

At 5% concentration Kiran *et al*., (2006) had demonstrated 66% inhibition in mycelial growth of *S.rolfsii* by *A. sativum* while present finding showed 100% inhibition in garlic at 4 & 5%. Compounds such as allicin (an oxide of diallyl disulphide) producing strong odour and ajoene present in garlic cloves might be responsible for its antifungal property (Cavallito and Bailey, 1950, Singh *et al*., 1990). Although Ginger was less effective on *S.rolfsii* but research showed 100% inhibition of ginger against root rot fungus (Suleiman and Emua, 2009). Sneha *et al.,* (2016) had showed that aqueous extracts of garlic and ginger in broth medium was 100% effective against *S. rolfsii*. However, the present finding showed poor inhibitory effect of synthetic fungicide like carbendazim. Efficacy of mancozeb against *S. rolfsii* was 100% while findings of Siddique *et al*., (2016) illustrated that carbendazim inhibited *S. rolfsii* @ of 74.44 %, while inhibition by mancozeb was 53.70%. Present finding demonstrated that garlic in combination with ginger {Garlic(2): Ginger(1) ,Garlic(3): Ginger(1) and Ginger(3): Garlic(1)} synergistically enhanced the inhibition in mycelial growth of *S.rolfsii* where garlic alone at 2% & 3% and ginger alone at 3% & 4% were not much effective.

Synergistic property in Garlic somehow goes with the finding of Daniel *et al*., (2015) who showed Garlic synergistically with low temperature inhibits growth of *Botrytis cineraria.* Moreover, Singh *et al*., (2003) also reported that the active components were antifungal individually but due to mixing in different combination, these active components showed strong antifungal efficacy against several sporulating fungi at very low concentration as compared to alone. The use of the combination of garlic and ginger and garlic alone in recent scenario will prove to be a great initiative to

sustainable agriculture system.

CONCLUSIONS

The present findings showed that Garlic could be more potent alternative to synthetic fungicides and Garlic in combination with ginger if incorporated in crop rotation system might greatly reduce the disease severity caused by *S. rolfsii* due to allelopathic effect. Use of Garlic as natural fungicide would also reduce the sole dependency on synthetic fungicides such as Bavistin.

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APPENDCIES

Figure 1: Southern Blight of Soybean Showing a. Field View of Southern Blight Affected Soybean Plants, b. Mycelial Proliferation on Stem of Soybean Plant, c. Disease Affected Pods of Soybean

Table 1, 2 & 3: Showing antifungal efficacy of garlic and ginger, their combination and comparison with synthetic fungicides such as Bavistin and Dithane M-45:

Table 1: Antifungal Efficacy of Garlic and Ginger Against Radial Growth of *S. Rolfsii.* Radial Growth (Inhibition %)

Plant Extracts	1%	2%	3%	4%	5%
Control	90±0.0 (0)	90±0.0 (0)	90±0.0 (0)	90±0.0 (0)	90±0.0 (0)
A.sativum	90±0.0 (0)	90±0.0 (0)	50.6±1.4 (43.7)	0.0±0.0 (100)	0.0±0.0 (100)
Z.officinale	90±0.0 (0)	90±0.0 (0)	80.6±4.0 (10.4)	74.0±3.5 (17.2)	43.6±3.4 (51.5)

Table 2: Antifungal efficacy of Garlic-Ginger Combination Against *S.Rolfsii* Radial Growth (Inhibition %)

Combination	1:1	2:1	3:1
A+Z	90±0.0 (0)	69.3±1.8 (23)	39±3.05 (56.6)
Z+A	90±0.0 (0)	90±0.0 (0)	69.6±0.8 (22.6)
Where, A= *A.sativum*, Z= *Z.officinale*			

Table 3: Antifungal Efficacy of Bavistin and Dithane-M45 Against *S. Rolfsii* Radial Growth (Inhibition%)

Conc.(in ppm)	Bavistin	Conc.(in ppm)	Dithane – M45
500	90±0.0 (0)	1500	0.0±0.0 (100)
750	90±0.0 (0)	2000	0.0±0.0 (100)
1000	90±0.0 (0)	2500	0.0±0.0 (100)
Where, Conc. = Concentration			

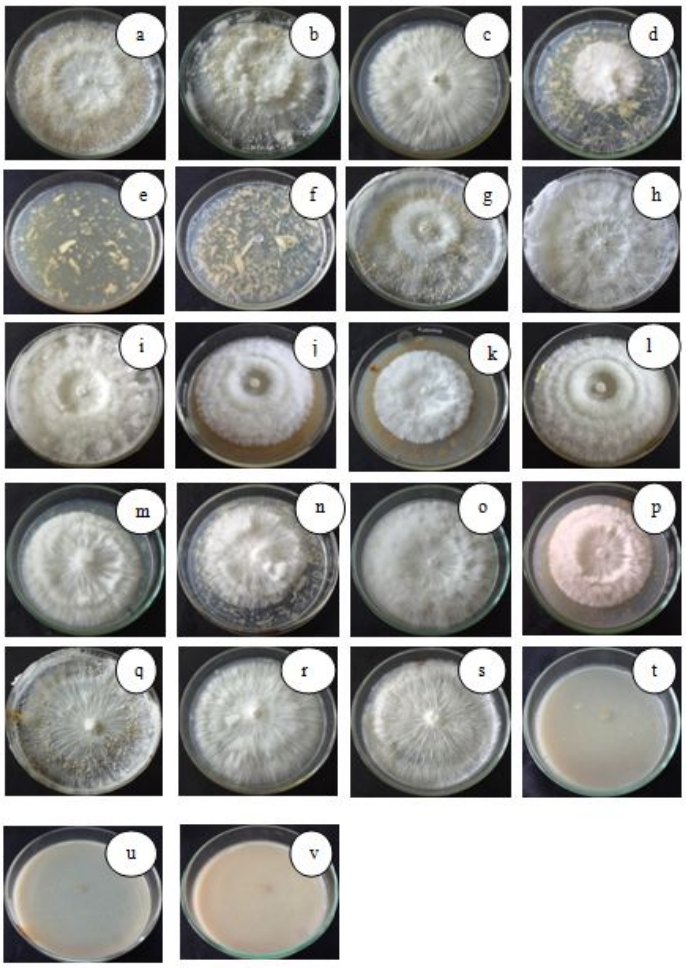

Figure 2: Showing the Radial Growth of S. Rolfsii Against Garlic, Ginger, in their Combination and Against Synthetic Fungicides with Respect to Control

a. Control, b. Garlic 1%, c. Garlic 2%, d. Garlic 3%, e. Garlic 4%, f. Garlic 5%, g. Ginger 1%, h. Ginger 2%, i. Ginger 3%, j. Ginger 4%, k. Ginger 5%, l. Garlic (1) : Ginger (1), m. Garlic (2) : Ginger (1), n. Garlic (3) : Ginger (1), o. Ginger (2) : Garlic (1), p. Ginger (3) : Garlic (1), q. Bavistin (50mg), r. Bavistin (75mg), s. Bavistin (100mg), t. Dithane-M45 (150mg), u. Dithane-M45 (200mg), v. Dithane-M45 (250mg).